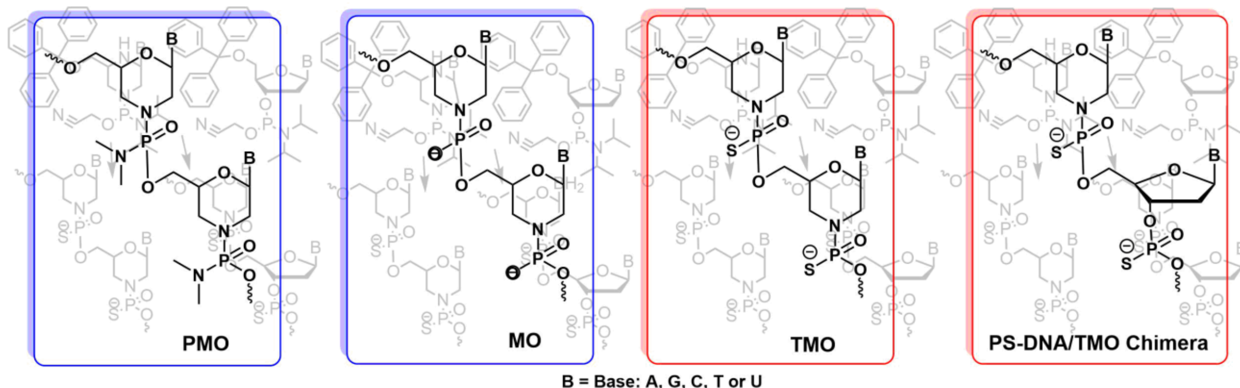
# Thiomorpholino oligonucleotides (TMOs) – An innovative class of Antisense oligonucleotide

Oligodeoxynucleotide, ODN analogues, are versatile biological and therapeutic agents that have various applications such as RNA interference, splice switching, aptamer binding, and antisense technology.[1] ODN's efficiency in delivery and gene silencing was improved tremendously by the introduction of GalNAc conjugation and lipid formulation. Antisense oligonucleotides (ASOs) have made significant progress in recent years and are beneficial for patients with both common and rare diseases due to their chemical diversity.[2]

ASOs are synthetic nucleic acid sequences that can specifically bind to certain RNA sequences using Watson-Crick base pairing. ASOs regulate the gene expression through steric blocking of RNA-protein interactions, RNase-H dependent cleavage or modulation of pre-mRNA splicing.[3] To be effective, ASOs require high nuclease resistance, high RNA targeting affinity, and better mismatch identification, leading to the development of chemical modifications on ASO that improve the pharmacokinetic profile of nucleic acid-based drugs. Since 1998, the FDA has approved nine ASO-based drugs, with many currently undergoing phase II and phase III clinical trials.[4] Some well-known chemical modifications on ASOs include 2'-OMe, 2'-O-MOE, 2'-F, PMO, LNA, PNA, tcDNA, BNA, and UNA.

PMO, phosphorodiamidate morpholino oligomers, is an important antisense agent that is designed by the replacement of ribose sugar unit of a natural nucleotide with a morpholino ring. Neutral backbone of the PMO offers low toxicity, water solubility, and sufficient endonuclease stability.[5] Four PMO-based drugs have been approved by the FDA to treat Duchenne muscular dystrophy patients, while three PMOs have shown their effectiveness against viral and bacterial infections and cancers in preclinical models and cell-based studies.[6] However, PMOs can be challenging to synthesize on a large scale for therapeutic use, they are not suitable for synthesis of hybrid morpholinos too.[7] Also, PMOs and other regular less toxic phosphorodiester backbone oligonucleotides are rapidly excreted from the body through Urine due to their poor binding to plasma proteins. Whereas thiophosphorodiester backbone or PS-DNAs improve the resistance and in vivo circulation time of ASOs by binding to serum albumin. Stabilin class of scavenger receptors bind effectively to thiophosphorodiester backbone.[8]




When the non-bridging oxygen atom in the phosphate backbone of morpholino phosphoramidates, MOs, are replaced by sulfur atom thiomorpholino phosphoramidates, TMOs are formed. A versatile solid phase synthesis of TMO and their DNA/TMO chimeras on an automated synthesizer have been developed by Caruthers et al using morpholino phosphoramidite as precursor.[9] Morpholino phosphoramidites were

prepared from 6’- DMT protected morpholinos with standard base labile protecting groups on the nucleoside bases. This chemical modification of TMO enhances their stability, resistance to nucleases, and binding affinity to RNA targets. Caruthers group reported that, compared to conventional DNA/RNA duplex, alternating PS-DNA/TMO chimeras and fully modified TMOs shows higher RNA binding affinity. Whereas fully modified TMOs were not recruiting RNase H1. Chimeric TMO analogues were efficient for high gene silencing compared to 2’-OMe control.[9a] Thus the DNA/TMO chimeras can degrade the mRNA by binding with RNase H and the high RNA affinity of fully modified TMO can block the gene expression and can be a good candidate for splicing studies.

Veedu et al. reported that compared to conventional PMOs, 2’-OMe and 2’-MOE, TMOs exhibit efficient exon 23 skipping in the mouse dystrophin transcript at a lower concentration of 5-20 nM. This can improve the drug safety profile by minimizing the dosage of the drug.[10] Rinn et al. reported that TMOs effectively block splicing and affect subcellular localization and availability of the RNA. TMO could be applicable not only to specific intron transcripts, but to variety of oncogene transcripts that could be performed inert in the nucleus.[11]

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